



**Steps for adding scale bars to images using Image J:**

1. Open Image J.
2. Click File, Open, Select the image you want to work with.
3. Click Analyze, choose "Set Scale."
4. Distance in Pixels is dependent on the magnification and type of image (optical or spectral). Use the table below to select the appropriate number.
5. Set the Known Distance and Pixel Aspect Ratio to 1.
6. Set Unit of Length to microns (um).
7. Check box next to Global. This will save your settings for subsequent images. Click OK.
8. Click Analyze, select Tools, click Scale Bar.
9. Scale bar will appear in lower right corner. Select scale bar length.
10. Click File, Save As. Save image.

Magnification	Optical (Retiga)	Spectral (Pixelfly)	Spectral (Andor)	3D (EXi Blue)
10x	2.20	0.79	0.625	6.45
20x	4.41	1.58	1.25	12.90
40x	8.81	3.17	2.50	25.80
50x	11.01	3.96	3.13	32.25
60x	13.21	4.76	3.75	38.70
100x	22.03	7.93	6.25	64.50